(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
22 April 2004 (22.04.2004)

PCT

(10) International Publication Number
WO 2004/033649 A2

(51) International Patent Classification[7]: C12N

(21) International Application Number:
PCT/US2003/031874

(22) International Filing Date: 7 October 2003 (07.10.2003)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/417,009 7 October 2002 (07.10.2002) US

(71) Applicant *(for all designated States except US)*: **UNIVERSITY OF MEDICINE AND DENTISTRY OF NEW JERSEY** [US/US]; 335 George Street, Suite 3200, New Brunswick, NJ 08901 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **LI, Honghua** [CN/US]; 24 Potomac Road, Monmouth Junction, NJ 08852 (US). **LI, James** [CN/US]; 24 Potomac Road, Monmouth Junction, NJ 08852 (US).

(74) Agent: **ZHU, James, J.**; Perkins Coie LLP, Patent - LA, P.O. Box 1208, Seattle, WA 98111-1208 (US).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *without international search report and to be republished upon receipt of that report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) **Title:** HIGH THROUGHPUT MULTIPLEX DNA SEQUENCE AMPLIFICATIONS

Criteria Used in Designing Primers That Are Experimentally Acceptable	
Tm Range of the primers (°C)	75-103
Length of the primers (bp)	24-33
Number of consecutive matching bases between the 3' ends of any two primer molecules	<4
Number of consecutive matching bases with one mismatch between the 3'-ends of any two primer molecules	<7
Number of consecutive matching bases between the 3'-end of one primer molecule and anywhere in another primer molecule	<9
Number of consecutive matching bases with one mismatch between the 3'-end of one primer molecule and anywhere in another primer molecule	<11
Maximal number of matching bases between two primer molecules	<75%
Number of consecutive matching bases between the 3'-end a primer molecule and anywhere in a sequence of a PCR product that is not the sequence to which the primer is designed to anneal to	12
Number of consecutive matching bases with one mismatch between the 3'-end of a primer molecule and anywhere in a sequence of a PCR product that is not the sequence to which the primer is designed to anneal to	15
Maximal number of matching bases between a primer molecule and a sequence of a PCR product that is not the sequence to which the primer is designed to anneal to	<80%

(57) **Abstract:** The present invention provides methods of designing PCR primers that allow the efficient and simultaneous amplification of a large number of different desired DNA fragments in a single multiplex PCR and minimize the formation of nonspecific extensions of undesired DNA fragments. The present invention allows a multiplex PCRto use at least 50 pairs of primers and produce at least 50 DNA fragments of interest. The present invention significantly broadens the application of multiplex PCR in the identification of multiple genes related to multifactorial diseases, the genome-scale detection of genetic alterations, the studies in large-scale pharmacogenetic reactions, the genotyping genetic polymorphism in a large population, the gene expression profiling in various samples, and high throughput genotyping technologies.

WO 2004/033649 A2